AN EVALUATION OF RICHARDSON'S VERIFICATION TEST IN THE SERO-DIAGNOSIS OF SYPHILIS

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The problems of the sero-diagnosis of syphilis have received much attention during the last few years, partly because of the wartime increase in the incidence of venereal diseases, but also because of the fashion of mass-testing recruits, blood-donors, immigrants and betrothed people. When testing on a large scale has been undertaken, it has become quite clear that the standard tests may frequently mislead the clinician, and that the results of these tests may often differ with the same serum.

In order to minimize these difficulties certain important recommendations have been made (League of Nations, 1932; Moore, Eagle & Mohr, 1940). Attention has been drawn to the technical errors both in withdrawal of, and in testing and reporting on specimens; the tests have been standardized and it is advised that a flocculation procedure and a complement fixation test be undertaken for each serum. Clinicians are further reminded to confirm every positive result by having a further sample of serum examined. However, even when these stringent conditions are satisfied, and when the reagents and techniques are constant and reliable, there remain still a number of serological results which do not help, and may mislead, the clinician in charge of the patients.

THE PROBLEMS

In the majority of cases, the serological results and the clinical diagnosis are perfectly straightforward. When difficulties are encountered, more noticeably in large-scale testing, the undecided cases fall into four categories:

(1) The failure of the Wassermann reaction (W.R.) or Kahn test, or both, to show a positive response in a case clinically believed to be syphilis.

(2) A discrepancy between the W.R. and Kahn results in cases exposed to infection, but clinically equivocal.

(3) A positive response with either or both tests in a person not exposed to infection or, at any rate, not suspected clinically of having contracted syphilis.

(4) A discrepancy between the tests in sero-reversal after treatment, often resulting in prolonged failure of one test to revert despite treatment believed to be adequate.

The terms W.R. and Kahn are to be considered as synonymous with complement fixation test and flocculation procedure respectively; in our experience the Kahn test is usually rather more sensitive and less specific than the W.R. These problems are now reconsidered severally in more detail.

(1) If the serum is examined very soon after exposure to infection the antibody response may still be inapparent or weak. Equally a doubtful positive result at this time may be due to non-specific causes. In such circumstances, and, if the clinical findings are equivocal, it may be necessary to delay treatment until a stronger serological response is obtained, an undesirable procrastination. The decision is further complicated when the patient has received treatment either for suspected syphilis or for coincident gonorrhoea. This difficulty has been accentuated by the advent of penicillin therapy (Heggie, Maguire, Bull & Heggie, 1947). We agree with Moore *et al.* (1940) that giving 'provocative' doses of arsenic does not clarify the doubts. Some other mechanism of increasing a weak specific result is required.

(2) The problem of a discrepancy between the tests on a person clinically equivocal is particularly difficult. It must be decided whether the positive result to one test is a non-specific response in a healthy individual, or whether the patient has syphilis and one test has failed to respond. The question here is one of specificity.

(3) Some patients who do not seem to have syphilis, and seem not to have been exposed to infection, may regularly provide a positive response to one or both tests. It should not be assumed that such non-specific responses are either transient or of low titre; it may be otherwise (Davis, 1944; Mohr, Moore & Eagle, 1941; Thompson, 1947). These cases are rare, but much anxiety and distress may result from an unjustified diagnosis of syphilis.

(4) The final and more common difficulty, is the failure to secure complete sero-negativity even for a long period after a course of treatment usually considered to be adequate. It is uncertain whether such cases represent a failure to eradicate the infection, a state of 'latent' syphilis. Perhaps the fact that many of these cases spontaneously revert to sero-negative many months after the treatment is finished is evidence on the other side. Jordan

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& Dolce (1946) followed up a series of such cases for 10 years and demonstrated marked fluctuations in the serological reactions.

All these problems are merely facets of the same difficulty, that the sensitivity and specificity of these tests are reciprocally interdependent. In either case, the sensitivity may be increased by modifying the technique, but the specificity decreases *pari passu*. So the tests, as at present used, aim at a practical balance between insensitivity and non-specificity, which gives excellent results in over 90 % of cases. Kolmer (1944) very rightly argues that over-sensitive techniques do more harm than good and recommends a 'practical sensitivity'.

GENERAL CONSIDERATIONS

Until the nature of the reacting substance present in the syphilitic person's serum is better understood, it will be difficult to devise more suitable tests. Recent opinions on the nature of this reacting substance are given by Weil (1941) and Sachs (1942). There is considerable evidence, adduced in different ways, that the syphilitic reacting substance is also present in very small quantities in many, if not all, normal sera (Hinkleman, 1927; Dreyer & Ward, 1923; Barnett, Jones & Kulchar, 1935; Sherwood, Bond & Canuteson, 1941; Lund, 1942). If this is so the difference between normal and syphilitic sera in this respect may be quantitative rather than qualitative (Malloy & Kahn, 1931). Inevitably, therefore, some weakly positive sera will fall outside the effective zone of any present test, and conversely some normal sera, containing an unusually large quantity of some non-specific reacting substance, will lie within it.

These latter non-specific positive sera have received some study. Eagle (1941) estimates the incidence in healthy people at about 1 in 4000. At this rate we would expect to encounter one such response about once in 3 months in our laboratory, and even more frequently at a Blood Transfusion depot. The incidence appears to be higher than this in hospital patients (Colquhoun, Kyles & Rannie, 1945) and this fact raises the question of the several diseases in which a number of non-specific positive Wassermann reactions are encountered. Apart from these diseases, there are other factors which are reported to influence the incidence of these reactions, such as pregnancy, or the season (higher in winter; Stokes, Boerner, Hitchens & Nemsler, 1946). There is also evidence that the content of the reacting substance in the serum varies from day to day (Greenbaum & Yagle, 1926; Nigg & Larsen, 1928). However, it cannot be said that non-specific positive results are always low in titre and transient. They may be both powerful and prolonged, as our own results and those of Mohr, Moore and Eagle show. Stokes *et al.* (1946) report that 78 % of non-specific positive results require longer than 3 months' observation before final evaluation is possible, and say, 'even at the end of a year, the diagnosis may be undetermined'. It must be borne in mind that a given serum may contain both specific and non-specific reacting factors.

THE VALUE OF CURRENT TESTS

Countless publications testify to the value of the tests at present in use, and it is generally agreed that both a flocculation procedure and a complement-fixation test should be performed on every specimen. The non-specificity and discrepancy rates vary very considerably in each of the reports in which figures are quoted (Dreyer & Ward, 1923; Endicott, 1927; Webb, 1936; Harrison & Osmond, 1943; Hartmann & Schøne, 1943; Berger & Sutherland, 1944; Colquhoun *et al.* 1945; Berger & Denton, 1944; Kolmer, 1944; Heggie *et al.* 1947; Vaughan, 1947; Price, 1948). In assessing the figures it should be remembered that variations must be expected, depending upon the type of population studied, e.g. healthy blood donors, or a venereal clinic, whether doubtful positives are ignored or included, and upon individual differences in technique and interpretation.

Generally speaking, it is agreed that the non-specificity rate for both tests should be less than 1 %. On the other hand, the discrepancy rate is incalculable and depends upon the number of tests employed; it is inevitably high if treated syphilitics are included. It is also generally held that flocculation tests are usually rather more sensitive and less specific than complement-fixation tests. The interpretation of a doubtful result may, indeed should, often differ depending upon whether the inquiry is concerned with diagnosis or assessment of therapeusis. For this reason, and because of the variables just mentioned we believe that statistical evaluation of tests is always misleading. Furthermore, the very small number of relevant discrepancies compared with the large number of tests involved renders the statistical significance of the deductions very doubtful.

It seems clear, therefore, that some improved or additional procedure is desirable which will assist in doubtful results and which should be judged without calculations involving large numbers of normal and satisfactory tests. It is possible that the solution may be found in an absorption technique using a cardiolipin antigen (Vogelsang, 1946) or through a conglutinating complement test (Hole & Coombs, 1947). A number of 'verification' techniques have been described (Hecht, 1921; Wassermann, 1921; Witebsky, 1938; Rytz, 1942; Kahn, 1940, 1943; Rein and Pillemer, quoted by Rein & Elsberg 1944,

1945) but, though we have been unable to find reports of any substantial trial of Witebsky's interesting elution technique, it appears that none of these sufficiently improve the specificity to be of value.

RICHARDSON'S TEST

In 1940 Richardson described a modification of Harrison and Wyler's technique for the Bordet-Wassermann reaction. Some preliminary results were published, but the investigation remained uncompleted on account of wartime restrictions.

The test has been in use, as a verification procedure only, in this laboratory for 7 years. It appears to assist in a number of doubtful cases and has the added advantage that no additional equipment or specialized serological knowledge is required.

Richardson describes the modification as 'the use of zone phenomena to distinguish specific from non-specific results'. The bases of the test are:

(1) To improve the sensitivity and to alter the proportions of the reagents in favour of the antibody, by diluting the serum only 1 in 2 (Wyler, 1932; Fairbrother, 1933).

(2) To improve the specificity and to alter the proportions further in favour of the antibody by diluting the finally constituted antigen 1 in 7.

This step should 'dilute out' coincident lipoid substances in the antigenic extract which are believed to give strong non-specific reactions with certain normal sera.

By these two alterations in the quantity of the reagents Richardson believes (*a*) that a specific reaction which may be partially suppressed by falling within an antigen excess zone in the normal test, is enhanced by the reagents being mixed more nearly in optimal proportions; and (*b*) that non-specific reactions are weakened by dilution of the non-specific lipoid fractions of the antigen. This test, therefore, aims at improving both the specificity and the sensitivity of the normal procedure.

There is a third modification incorporated by Richardson, that of the addition of a one-fifth volume of 0·1 % magnesium chloride. It is true that small concentrations of magnesium are necessary for specific immune haemolysis, and the addition of the chloride or sulphate in a strength of not more than 0·05 g. %, will increase the speed of haemolysis. When concentrations of 0·3–0·5 g. % are reached, however, the effect is to inhibit haemolysis (Cernovodeanu & Henri, 1906; Kelogg & Wells, 1926; Mayer, Osler, Bier & Heidelberger, 1946). Since the initial magnesium content of each sample of serum and reagents remains variable and undetermined, the selection of a one-fifth volume of 0·1 % chloride salt may seem rather empirical.

Though the influence of varying concentration of magnesium ions should be carefully determined, it is at present our view that the first two modifications alone are responsible for the characteristic properties of the test.

TECHNIQUE

The methods employed have been constant for more than 7 years, and there has been no occasion to suspect irregularity or variation in the results. The reagents employed were regularly standardized, and controlled in the usual manner. The complement used was a reconstituted dried product, 'Lyovac'. The complement-fixation method was exactly that of Harrison & Wyler (Wyler, 1929; modified in 1932) except that (*a*) the sensitized cell suspension was regularly prepared on the day previous to the test and stood overnight at 4° C., and (*b*) only 2 M.C.D. strengths were used in the control tubes. The Kahn antigen was that distributed by the Ministry of Health, and the standard procedure was followed, except that in cold weather the tubes were stood for 20 min. at 37° C. before adding the final saline volumes and reading the results. The reasons for these modifications need not be discussed, but it may be said that the results appeared to be satisfactory and constant.

In reading the results, the following symbols were used for the W.R.: + +, complete fixation of 3 and 5 M.C.D.; +, complete fixation of 3 M.C.D. only; ±, partial fixation of 3 M.C.D., half an hour after the control tube had cleared. For the Kahn: + +, marked flocculation in all 3 dilutions; +, marked flocculation in 2 dilutions; ±, marked flocculation in the weakest dilution only.

Each batch of sera, averaging 150 in number, was tested over a period of 2 days. On the first day the Kahn tests were done, and the haemolytic system prepared. The Wassermann reactions were done the following morning, after preliminary standardization of the reagents. Any sera giving equivocal or unexpected results were retested by Richardson's modification on the same afternoon; about twelve such sera were usually treated in this way from each batch. These sera were subjected to a three-tube test; one tube being a serum control, another Richardson's modification, and the third, a repetition of the 3 M.C.D. Wassermann reaction as additional evidence against possible deterioration of the reagents since the morning. The three tubes were set up as shown in table on p. 266.

The volumes are in ml.; they are measured by Donald's droppers standardized with a Starrett wire gauge. A no. 56 dropper delivers a volume of 0·022 ml. per drop of an aqueous solution; 0·11 ml. is given by two drops from a no. 30 dropper for aqueous solutions, and by two drops from a no. 18 dropper for alcoholic solutions. The diluted antigen is treated as an aqueous solution.

	Serum	Saline	0·1% $MgCl_2$	(3 M.C.D.) complement	Antigen	Diluted antigen
Row 1. Control	0·044	0·044 + 0·11	0·022	0·11	—	—
Row 2. As W.R.	0·022	0·11	—	0·11	0·11	—
Row 3. Richardson	0·044	0·044	0·022	0·11	—	0·11

The test is thereafter carried out as the Wassermann reaction. Absence of haemolysis is read as positive; complete haemolysis as negative, and partial haemolysis as doubtful. The last finding is very rare.

RESULTS

During the course of about 18 months nearly 20,000 sera were examined by the W.R. and Kahn tests. Of these, about 1500 were submitted to Richardson's modification as a verification procedure. A number of cases (ninety-two in all) on whom this test has been performed have been followed up, and form the material on which this paper is based. For reasons given above, we do not believe that a statistical analysis involving large numbers of normal individuals and treated cases inseparably mixed can yield information of positive value. Our sera being drawn from such mixed sources, we have accordingly confined our analysis to those cases in which doubtful serological and clinical findings called for a verification procedure. An attempt is made to assess whether Richardson's modification does in fact prove of value in such cases.

In an untreated case of syphilis the Kahn reaction usually becomes positive first, and later Richardson's test, and finally the standard W.R. The return to sero-negativity is generally in the reverse order.

A man reported with a typical penile sore in which treponemata were demonstrated by dark-ground illumination. Specimens received at the laboratory on the dates mentioned gave the following results:

1. iii. 47	Kahn	+	W.R.	−	R.	−
19. iii. 47	Kahn	+	W.R.	−	R.	+
26. iii. 47	Kahn	++	W.R.	+	R.	+

This is a typical response, though occasionally there may be exceptions.

With this natural response in mind, the results are presented in groups. The test has been applied in some cases when the Kahn has been positive, but the W.R. negative (Group I). On the occasion, too, when the W.R. is positive and the Kahn negative (Group II) the test has been used. There is also a small number of cases when Richardson's test was positive, though the standard tests were negative (Group III).

Group I

The common diagnostic discrepancy is a positive Kahn and a negative W.R. from a person recently exposed, or at any rate untreated. Fifty-nine cases have been included in this group; Richardson's test being applied on each serum. In twenty-four the test was positive, and twenty-two of these cases subsequently proved to have syphilis; the criteria of diagnosis accepted being (*a*) demonstration of treponemata by dark-ground illumination, or (*b*) a history of exposure, a subsequent full positive serological response, and either a genital sore or other unequivocal clinical evidence of the disease. Of the remaining thirty-five cases in which the test was negative, thirty proved not to have had syphilis but five were thought to be so infected. One way of grouping these results is shown in Table 1.

Table 1

No. of cases	Richardson's Test	Final diagnosis
22	+	Syphilis
2	+	Not syphilis
30	—	Not syphilis
5	—	Syphilis

It would seem that, in the diagnosis of those cases in which the Kahn is positive and the W.R. negative, the modification of Richardson affords valuable corroborative evidence.

When the test is positive, nearly all the patients subsequently prove to have syphilis and from the twenty-two cases four are selected for brief individual mention.

Case 1 presented himself with ulcers of the legs of uncertain origin; the Kahn test and Richardson's test were strongly positive, but the W.R. was negative on 17 May 1947. A week later all three tests were positive and remained so until a course of treatment reversed the serology and improved the condition.

Case 2 reported with balanitis and a history of recent exposure. On 12 April 1947 both Kahn's and Richardson's tests were positive, but the W.R. was negative. By 8 May 1947 a fully positive serological response had developed.

Case 3 was diagnosed as aneurysm of the aorta. The Kahn was doubtful and the W.R. was negative. Richardson's test was positive. Fifteen years earlier the patient had been exposed to syphilitic infection. It may be said that for this case serology need have played no part in the diagnosis, but it is interesting that the doubt due to an uncertain response to one test only, may be removed without the delay involved in awaiting a further specimen.

Case 4. A man presented with a penile sore of uncertain nature on 12 July 1947 when the W.R. was negative and the Kahn test was strongly positive. On 30 July 1947 the results were similar, and Richardson's test was positive; the W.R. did not become positive until 13 August 1947. The use of Richardson's test in this case implies that treatment may be applied in good time, even if the Wassermann response is delayed.

In two cases, however, the positive response to Richardson's test was in error. It can be seen from the histories that a diagnosis of syphilis would not have been entertained in either case, but the results are quoted so that the evidence may be complete.

Case 5. This man had a pronounced fear of contracting syphilis. Though he reported a recent exposure, he had no signs or symptoms of the disease. The W.R. was negative on the first occasion of testing the serum (17 May 1947), but the Kahn response was doubtfully positive and Richardson's test was positive. It was noted, however, that the serum was not in good condition, and the result was suspect. When repeated on 28 May 1947, all the tests were negative, as they were on subsequent occasions. The cerebro-spinal fluid was normal.

Case 6. A routine serum examination was performed on a pregnant woman, who was believed to be in good health. The W.R. was negative, but the Kahn test was doubtfully positive, and Richardson's test was positive (26 March 1947). On 9 April 1947 the tests were repeated, and were all negative. A healthy infant was born 20 October 1947. The cerebro-spinal fluid was normal.

Of those cases which gave a negative response to Richardson's test in this group, a few were conditions in which syphilis was not likely, though the Kahn had shown an unexpected and probably non-specific positive. These included such conditions as infected haemorrhoids (mistaken for condylomata), tuberculosis, tympanic sclerosis, dental cyst, skin rashes and pregnancy. A number, however, had atypical penile sores or gonorrhoea, and in such cases a positive Kahn may be confusing to the clinician.

In three cases the false positive Kahn reaction was both strong and prolonged, and they deserve mention on account of their intrinsic interest.

Case 7 was a female referred to the venereologist because a strongly positive Kahn response had been found at the Blood Transfusion centre. She had no signs or symptoms of syphilis and denied any risk. On examination she appeared to be a virgo intacta. In December 1947 her serum was examined and a strongly positive Kahn response obtained, though both the W.R. and Richardson's test were negative. An identical serological response was obtained in January, March, July and September of the following year, a period of 9 months in all. She was observed over this time and appeared to be in good health. The serological results were identical when serum was sent to other laboratories for confirmation. The cerebro-spinal fluid was normal. The patient returned to Ireland and no further information was available. It is unlikely that she had syphilis, yet Kahn response alone was both prolonged and powerful.

Case 8 was similar. A female reported on 8 November 1947, with a rash. At this time the Kahn was strongly positive, but the W.R. and Richardson's tests were negative. She denied any risk of infection and on examination appeared to be a virgo intacta. No signs or symptoms of syphilis developed over the observation period of just over a year. During this time, however, the same serological response was regularly obtained both in this and other laboratories. Price's precipitation reaction (Price, 1948), however, was negative. The cerebro-spinal fluid was normal.

Case 9, showed the same results over a shorter period. A male sought a premarital examination, a request which consorted ill with his denial of exposure to infection. He feared contraction of syphilis through fomites. He had no signs or symptoms of the disease and his cerebro-spinal fluid was normal. The Kahn was strongly positive to weekly serological tests for a period of 2 months, but the W.R., Richardson's test and Price's precipitation reaction were negative. These results were confirmed in other hands.

These cases are quoted to show that non-specific reactions need not be weak or transient. In such cases, were the history less conclusive, it would be easy to assume that the response was specific and that the W.R. had failed to react, or had been masked by treatment undeclared by the patient, perhaps for some other condition. The test under review affords valuable corroborative evidence in such problems.

Richardson's test, however, was negative in five cases of this group, and they were, nevertheless, syphilitic. Here the problem is merely one of time. It can be seen from the example given on p. 266, that there is normally a period in the syphilitic patient during which the more sensitive Kahn has responded before Richardson's test, which itself is more sensitive than the W.R. Richardson's test, therefore, merely assists by narrowing down rather than completely closing the gap, and should be considered in that light. Indeed, of the five discrepant cases from Table 1, four were justifiably treated on the basis of clinical findings and the strongly positive Kahn, so that neither complement fixation test became positive at any time. These four cases therefore add statistical weight against the test which is not justified in practice. The fifth case, however, showed a failure in sensitivity of Richardson's test, and is briefly recorded.

Case 10. This man, after exposure, reported with a condition resembling urethritis. His serological findings read as follows:

29. i. 47	Kahn	+	W.R.	–	R.	–
5. ii. 47	Kahn	+	W.R.	+	R.	–
12. ii. 47	Kahn	+	W.R.	–	R.	–
19. ii. 47	Kahn	+	W.R.	–	R.	–
27. ii. 47	Kahn	+ +	W.R.	–	R.	–
2. iv. 47	Kahn	±	W.R.	–	R.	–
12. iv. 47	Kahn	–	W.R.	–	R.	–

The diagnosis of syphilis was made, though never conclusively proved, on clinical grounds, and treatment started on 5 February 1947. However, on that date, though the W.R. was positive, Richardson's test remained negative. It can be seen that the serological results reverted in due course.

In considering the results in this group, it must be remembered that Richardson's test was intended to clarify doubtful Wassermann results. In this group the range of the test has been extended to serve in solving the problems raised by doubtful Kahn results, a rather more difficult task. Furthermore, it must be remembered that the cases quoted are only those in which some element of doubt or discrepancy occurs. Nevertheless, of fifty-nine such cases, Richardson's test responded specifically in fifty-two. In our view, therefore, the test does contribute in good measure to the solution of this type of problem. A careful consideration of the seven cases in which the test was misleading shows that usually the clinical findings are not in doubt. The two non-specific positive results (cases 5 and 6) were clinically assessed without difficulty. Of the five negative results, one (case 10) gave rather inconclusive evidence, and the remaining four were merely negative because rigorous treatment was applied before full sero-positivity had had time to develop.

Group II

The alternative discrepancy is when the W.R. is positive and the Kahn test negative. It is necessary to decide whether the W.R. is showing a non-specific response, or whether it is one of those unusual occasions when the complement-fixation technique is proving more sensitive than a flocculation procedure.

Twenty-eight cases fall into this group, of which five were positive by Richardson's test, all subsequently proving to have syphilis, and twenty-three were negative, and these latter were all thought not to have had syphilis. In this small group it appears that the test was invaluable as a verification procedure.

The following case is an example of the confirmation of a doubtful W.R. in a syphilitic patient:

Case 11, after exposure to infection, reported with a condition primarily diagnosed as balanitis. On 11 June 1947 the Kahn was negative and the W.R. doubtful. Richardson's test on this occasion was positive. Fully positive serological response developed on 28 June 1947 and was confirmed on 9 July 1947.

When Richardson's test was negative under these circumstances, the diagnosis invariably proved to be not syphilitic. The twenty-three cases in which a non-specific positive W.R. was found include such conditions as: orchitis, pregnancy, rheumatic fever, epilepsy and perforated nasal septum. In addition, there were some people in normal health, and several with gonorrhoea or atypical penile lesions. In every case the diagnosis of syphilis was satisfactorily excluded. In our view, this is a very important advance on routine testing methods and may save much anxiety in healthy patients otherwise laid under suspicion.

An example of such a case is given below:

Case 12, a female, reported for investigation after intercourse with an unknown consort. She had no signs or symptoms of syphilis at this time, or up to 19 months later. The W.R. was weakly positive, the Kahn doubtfully positive, but Richardson's test negative. Three weeks later all the tests were negative, but a further fortnight later the W.R. again gave a weak positive response. No treatment was given, and the patient remained in good health. All subsequent serological tests were negative.

There are also included in this group several cases in which the non-specific W.R. was strongly positive, and several in which the response was prolonged over many weeks. There are also a few cases in which both W.R. and Kahn showed a transient non-specific response at the same time. In all these cases Richardson's test was negative and syphilis was confidently excluded clinically.

In this group of twenty-eight cases in which the W.R. responded first, Richardson's test proved sensitive and specific without exception. There is no doubt that it is an invaluable confirmation test in such circumstances.

Group III

There remains a group of five cases in which Richardson's test was found to be positive, though both the W.R. and Kahn tests were negative. Of necessity this group must be small, since Richardson's test was carried out in general only on those sera which showed discordant results. However, when the serology appeared to conflict markedly with the clinical findings, the test was sometimes applied. In five such cases syphilis was strongly suspected, the routine tests were negative, Richardson's test was positive and the diagnosis subsequently confirmed. This is well illustrated by the following reports:

Case 13. A man presented himself with a typical penile sore on 23 April 1947, when both the W.R. and

Kahn tests were negative. Three days later the routine tests were repeated and were again negative, but Richardson's test was positive. A week later both the W.R. and Kahn tests were weakly positive and treponemata were demonstrated by dark-ground illumination. A fully positive serological response had developed by 2 July 1947.

Case 14. A man reported with a typical penile sore on 3 January 1948, but no treponemata were demonstrated by dark-ground illumination; the W.R. and Kahn tests were negative on this date, but Richardson's test was positive. A week later strongly positive W.R. and Kahn results were obtained.

In this small group the sensitivity of this test is well illustrated, and the serological confirmation of clinical suspicion is thereby hastened.

DISCUSSION

In assessing the value of a verification test, it is important that results should be considered when the test is applied only to those sera which give inconclusive results by the routine techniques. Surveys which include large numbers of normal and satisfactory results merely serve to dilute the essential results with irrelevant figures and in any event are questionable statistically. Vaughan (1947) has assessed the value of Richardson's test in this way, and presents figures for the specificity rate of this, and the routine tests, but without establishing criteria for the diagnosis of the disease, or presenting protocols of individual cases. Jennison, Penfold & Roberts (1949) have undertaken a statistical survey of the results obtained by Richardson's test in comparison with the standard W.R. on a thousand cases. The figures presented confirm the improved specificity and sensitivity of the test. The data, however, are purely comparative and, since the cases are not re-tested or followed up, the classification into groups is necessarily arbitrary.

We have been unable to find many reports which concern themselves with the ultimate diagnosis of cases which are equivocal serologically or clinically. Becker (1947) gives details of seventeen cases; twelve of these are fairly conclusive without the aid of a verification test, and the others are rather equivocal. Hodel (1942) describes the serological fluctuations of a patient who had not contracted syphilis, but in whom non-specific positive results, sometimes very strong, were given by both flocculation and complement-fixation tests, irregularly over a period of 4 years. Mohr *et al.* (1941) quote nine interesting cases of persons clearly not syphilitic, who gave a non-specific positive response either to flocculation or to complement-fixation tests, or to both. Two of these cases remained positive for about 2 years, and two other cases had quite high titres. These make an interesting comparison with our cases 7, 8 and 9. In our view neither the strength nor duration of a positive response is an index of specificity.

We have accordingly followed up ninety-two cases in which the serological results conflict, either between themselves, or with the clinical findings, and Richardson's test has given a reliable and accurate result in eighty-five cases. The test was designed to verify a doubtful response to the W.R., but the technique has been extended in this survey to cover all serological and clinical discrepancies. Considering that the cases under review are those which, by routine methods, would give results regarded as inconclusive or invalid, we believe that the figures presented show that the test may play a useful and reliable part in the solution of these problems.

In thirty-seven cases of proven syphilis Richardson's test was negative on five occasions; and in fifty-five cases unlikely to have been syphilis it was positive on two occasions. The results also indicate that a positive result in this test appears to be a slightly more reliable confirmatory response than a negative result. It is also noteworthy that, as a confirmatory test for the W.R., it was reliable in all of the twenty-eight cases studied in this group; the seven unreliable results were all encountered when the test was used to clarify a doubtful or unexpected Kahn response. On no occasion in about 1500 tests has this technique given an isolated false positive result.

In reconsidering the four problems reviewed above, Richardson's test plays no useful part in the solution of the fourth. In the other three problems, however, the test has given a reliable and helpful result in eighty-five of ninety-two cases. One disadvantage of the test is that the stronger serum dilution increases any anti-complementary activity of the serum, so that it has no application to W.R. results inconclusive on this account.

Any laboratory performing the Wassermann reaction as a routine can undertake this test with little extra trouble; no special skill or additional equipment are required. Used as a verification test it appears to give valuable evidence in cases difficult to assess and, we believe, should be more widely employed. We, therefore, cannot subscribe to the opinion, so frequently appearing in medical literature, that no verification tests in current use are of value.

SUMMARY

The importance of the accuracy of serological tests for syphilis has become more than ever emphasized by widespread testing and more efficient therapy. The problems are reviewed in the light of present knowledge of the specificity and sensitivity of standard serological tests for syphilis. Richardson's modification of the Wassermann reaction is de-

scribed. The results of this test when applied to ninety-two cases giving doubtful or discrepant reactions by routine tests are presented and classified. The cases were followed up clinically. The results indicate that this simple test is a reliable and valuable adjunct to routine methods, and should be employed widely as a verification procedure.

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